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THE UPTAKE OF STRONTIUM AND CALCIUM FROM SOILS BY GRASSES AND LEGUMES AND THE POSSIBLE SIGNIFICANCE IN RELATION TO SR-90 FALLOUT[1]

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INTRODUCTION

AN ATOMIC BOMB explosion results in the release of a substantial amount of radioactive material into the stratosphere, the subsequent fallout being world wide. Of the radioactive fission products released, cesium-137 and strontium-90 give most cause for concern, due to their long half-lives, 27 and 28 years, respectively. Of these two the former is less hazardous, as greater amounts are fixed in the soil in a form unavailable to plants (Nishita *et al.*, 1956)[4], while if ingested by animals it is incorporated into soft tissue and is more rapidly excreted (Hamilton, 1947; Hood and Comar, 1953). Strontium-90, however, appears to remain, at least in part, in the soil in a form available to plants (Klechkovsky and Tselishcheva, 1957; Schulz *et al.*, 1958) and is taken up by them similarly to calcium (Collander, 1941).

Animals feeding on contaminated herbage will absorb radioactive strontium (Gross *et al.*, 1953), which is retained in the skeleton and laid down in a manner similar to calcium (Hamilton, 1947). However, it is known that the absorption of strontium from the intestine is much less efficient than the absorption of calcium, while strontium excretion in the urine is more efficient (Harrison *et al.*, 1955). Similarly it has been found in dairy cows that although a proportion of the strontium ingested is excreted in the milk, there is a preferential excretion in favor of calcium, the ratio of strontium to calcium in milk being 0.10 to 0.14 of that in the diet (Comar *et al.*, 1957).

Radiostrontium is ingested by humans either by the direct consumption of contaminated vegetables or from milk, and the latter source is probably the most significant in a long-term view. Babies and young children are particularly vulnerable, both because of the large amount of milk they drink and their very active bone formation. Although at the present levels of radioactivity no immediate risk is foreseen, one or two instances are

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[4] See "Literature Cited" for citations referred to in the text by author and date.

known (Bryant *et al.*, 1958) of babies' bones having an activity of over 3.0 S.U., this level being just under a third of the level the Medical Research Council suggested might give cause for consideration if exceeded. The long-term genetic effects of increased general levels of radiation have been widely discussed (Muller, 1951, 1956; Mather, 1952; Ford, 1952; U. S. Govt., 1957). It may be noted that no precise information is yet available on the long-term physiological effects of continuous low-level strontium-90 intake (Anonymous, 1958).

In view of the nature of much of the evidence (U. S. Govt., 1957; Failla, 1958; U.N. Report, 1958; Russell, 1958) on which future world-wide radioactivity levels have been predicted, and also the possibility of increased bomb testing reactor accidents, or even atomic warfare, it seemed desirable to determine if species differences existed in forage plants, such that some might take up less strontium from the soil than others. This knowledge might then be used either in reducing the radiostrontium level in milk, or possibly in choosing species for reclaiming heavily contaminated land. That wide differences existed between species in their ability to remove strontium from the soil seemed clear from existing survey work (Bowen and Dymond, 1955), but precise comparative data of the strontium content of forage species grown under the same conditions on identical soils were lacking. A preliminary report has been published (Vose and Koontz, 1959).

TABLE 1

THE STRONTIUM AND CALCIUM CONTENT AND pH OF THE SOILS USED IN THE EXPERIMENT

Soil	μe Sr/ 100 gm	μe Ca/ 100 gm	Sr/Ca ratio ($\times 10^5$)	pH
Nord fine sandy loam	52.8	17,260	306	7.4
Yolo fine sandy loam	23.8	7,500	317	6.9
Sacramento fine sandy loam	17.6	4,460	395	7.0

EXPERIMENTAL

Sixteen species and strains of forage legumes and grasses were grown on three different soils, similar in texture and pH but varying in strontium and calcium content (table 1). Seedlings were germinated in sand and were transplanted to one-gallon pottery crocks when the grasses were at the second-leaf stage and the clovers showed the first trifoliate leaf. Ten seedlings were planted per crock in order to give complete cover. The plants were grown outdoors in triplicate and were watered throughout with distilled water, no fertilizer being added.

It was decided not to add either radioactive or stable strontium to the soil, partly because of the difficulties of adequately mixing small amounts of material into a large bulk of soil, and partly because it was felt that the very small amounts of native strontium in the soil would give a better picture of the uptake of minute amounts of strontium by plants. The behavior of stable strontium and Sr-89 and Sr-90 should be identical.

Harvesting was carried out at about eight weeks, the plants being grown

until they had reached the stage of incipient flowering, or in the case of those strains having a "winter requirement" for flowering, until they had reached maximum vegetative growth. The foliage was cut to within 2 cm of the ground, rinsed in distilled water to remove earth and dust, dried at 70° C, and ground in a small Wiley mill. Strontium was analyzed by X-ray emission spectrography, the instrument used being a General Electric Model XRD-5 maintained in the Department of Soils and Plant Nutrition.

The general procedure described by Brandt and Lazar (1958) for the analysis of zinc and molybdenum in dried plant material was used, the line/scatter ratio of an element being a measure of its concentration. However, in the case of strontium it is not possible to prepare a working curve from chemically analyzed samples due to the difficulties of chemical analysis (Bowen and Dymond, 1955).

The first attempt to prepare plant samples for irradiation was made by co-precipitating strontium and calcium as the oxalate from plant digests, the precipitate being collected on filter paper disks and dried with acetone. Standards were similarly prepared from pure strontium and calcium salts, and a working curve prepared. This method was found to be inaccurate as the large amount of calcium caused interference, and there were differences due to variation in sample thickness.

The method finally adopted was to present the ground plant material to the X-ray beam as a sample of infinite thickness. Standards were prepared by making known additions of strontium to ground plant material, the procedure being to thoroughly moisten the dry material with acetone, add the required strontium aliquot from a pipette, then thoroughly mix and stir. The paste was then placed on a steam bath until the acetone had been driven off, and placed in the oven overnight at 70° C. The dry sample was broken down into powder, using a pestle. Mixing occurred at every stage, and separate subsamples of each standard showed good reproducibility. When the line/scatter ratios of a number of standards were plotted, a straight line resulted, which could be extrapolated to obtain the strontium content of the original plant material. This is shown in figure 1, and it may be seen that standards based on different plant species resulted in parallel lines. It was thus possible to calculate a constant factor to enable the determination of the strontium content of any plant sample. In the present instance the mean increase in F/S ratio per 100 μg of added strontium for various plant material was 0.383 F/S unit.

Therefore, 1 μg Sr = 0.00383 F/S unit
and 1 μe Sr = 0.168 F/S unit

Thus for any plant material:

$$\frac{F/S - 1}{0.168} = \mu e \text{ Sr per gm}$$

The soils were extracted with ammonium acetate-ammonia at pH 9.0, this reagent having been found (Bowen and Dymond, 1956) to extract calcium and strontium from soil in approximately the same ratio as found in the plant. Strontium was analyzed by X-ray spectrography after aliquots of soil extract (in place of the strontium standard) were added to plant material of known strontium content. The soil strontium was found by difference.

TABLE 2

THE STRONTIUM AND CALCIUM CONTENT OF PASTURE SPECIES GROWN ON THREE DIFFERENT SOILS

Species	Soil											
	Nord fine sandy loam				Yolo fine sandy loam				Sacramento fine sandy loam			
	Sr μe/gm	Ca μe/gm	Sr/Ca ratio ($\times 10^5$)	OR*	Sr μe/gm	Ca μe/gm	Sr/Ca ratio ($\times 10^5$)	OR*	Sr μe/gm	Ca μe/gm	Sr/Ca ratio ($\times 10^5$)	OR*
Grasses:												
Bromus inermis, Manchar	1.49	348	428	1.4	0.83	202	411	1.3	1.25	306	408	1.0
Lolium perenne, Oregon Commercial	1.31	366	358	1.1	0.74	145	510	1.6	0.89	258	345	0.9
Lolium perenne, S. 101	1.19	390	305	1.0	0.71	165	430	1.4	0.89	249	357	0.9
Festuca elatior, Late Otofte	1.19	352	338	1.1	0.65	136	478	1.5	0.89	240	371	0.9
Festuca arundinacea, Alta	1.07	306	350	1.1	0.71	141	504	1.6	0.89	202	436	1.1
Phleum pratense, American Commercial	1.01	235	430	1.4	0.59	112	527	1.7	0.59	131	450	1.1
Phalaris tuberosa, Harding-grass	0.95	352	270	0.9	0.71	151	470	1.5	0.71	225	316	0.8
Dactylis glomerata, Potomac	0.93	235	396	1.3	0.65	141	461	1.5	0.77	198	389	1.0
Legumes:												
Trifolium subterraneum, Tallarook	4.70	1500	313	1.0	3.99	1050	380	1.2	4.22	1130	373	0.9
Medicago sativa, Caliverde	3.81	995	383	1.2	2.44	680	359	1.1	2.65	812	326	0.8
Trifolium fragericum, Salinas	3.57	1105	323	1.0	2.20	573	384	1.2	2.74	780	351	0.9
Trifolium repens. S. 100	3.51	1020	344	1.1	1.96	521	376	1.2	2.62	755	347	0.9
Trifolium repens, Dutch White	3.27	995	329	1.1	2.84	770	369	1.2	3.45	985	350	0.9
Trifolium pratense, Kenland	3.20	940	340	1.1	2.47	667	370	1.2	3.99	1130	353	0.9
Trifolium repens, Ladino	3.09	976	317	1.0	2.74	770	356	1.1	3.57	1050	340	0.9
Lotus corniculatus, Los Banos	2.53	718	352	1.1	1.67	390	428	1.3	2.38	648	367	0.9

* OR (Strontium–calcium observed ratio) = $\frac{\text{Sr/Ca in plant}}{\text{Sr/Ca in soil extract}}$

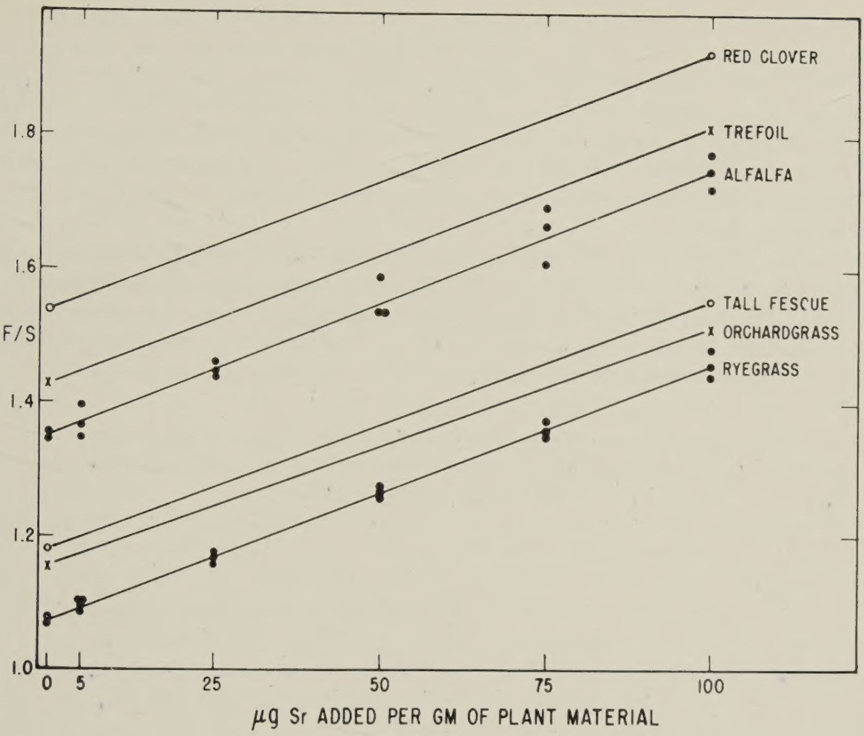

Fig. 1. Ratio of the strontium Kα (first order) radiation intensity to the scatter radiation intensity as a function of added amounts of strontium to dried, ground plant material of six species. (The alfalfa and ryegrass samples used in this figure have no relation to the plants used in table 2).

Calcium in both plant material and soils was determined by potassium permanganate titration of the precipitated oxalate (Piper, 1942).

RESULTS AND DISCUSSION

The results (table 2) indicate five significant features: 1) Every legume species takes up more strontium than any grass—three to six times as much in many cases. 2) Within either the grasses or the legumes the variation in strontium content between species and varieties is not marked. The range is slightly greater in the legumes than in the grasses, and the variation is greatest in material grown on soils with high extractable strontium. 3) The amount of strontium taken up is directly related to the calcium taken up. 4) The legumes grown on the two soils lowest in calcium have in general a Sr/Ca ratio a little lower than that of the corresponding grasses, but this trend is more than offset by their much greater actual strontium content. 5) The Sr/Ca ratios of the soils are of the same order as the Sr/Ca ratios of the plant material, and confirm the choice of ammonium acetate-ammonia at pH 9.0 as the soil extractant. In the case of plants grown in soil, the strontium/calcium observed ratio, OR, (Comar *et al.*, 1957) must be primarily a reflection of the soil extraction procedure, but it is interesting to

note that on the Yolo soil the grasses have a higher OR than the legumes, and also higher than either grasses or legumes on the other two soils. Work involving the uptake of five different ratios of radioactive strontium and calcium from solutions over a 24-hour period (Koontz and Vose) substantiates the idea that on some soils grasses, as opposed to legumes, will discriminate to some extent in favor of strontium. It is clear, however, that there is no major discrimination between strontium and calcium by either grasses or legumes.

Neither the grasses nor the legumes can be ranked in the same order of strontium content for every soil, but *Bromus inermis* and *Trifolium subterraneum* are consistently highest in their respective classes. Of the legumes, *Lotus corniculatus* has the least strontium, while *Phleum pratense, Dactylis glomerata,* and *Phalaris tuberosa* are the lowest ranking grasses.

TABLE 3

A GENERALIZED COMPARISON OF POSTULATED Sr/Ca RATIOS OF MILK FROM COWS FED GRASSES OR LEGUMES WITH AND WITHOUT A CALCIUM SUPPLEMENT

Diet	Forage (μe/gm DW)			Milk			
	Sr	Ca	Sr/Ca ($\times 10^5$)	Sr* (from forage)	Ca (from supplement)	Ca (from forage + supp.)	Sr/Ca ($\times 10^5$)
Grass	0.70	175	400	0.07	0	175	40.0
				0.07	625†	800	8.75
				0.07	1,250‡	1,425	4.9
Legume	3.2	800	400	0.32	0	800	40.0
				0.32	625	1,425	22.4

* The ratio of strontium to calcium in milk is taken as 0.1 of that in the diet.
† Sufficient to bring the Ca level of grass diet to the level of legume without supplement.
‡ Sufficient to bring the Ca level of grass diet to the level of legume with supplement.

This work has been carried out with stable strontium and certain reservation must, therefore, be placed on transposing the conclusions to the uptake of Sr-90. There is, however, no reason to suppose that the behavior of radioactive Sr-90 does not follow that of the stable isotope, but it must be realized that usually only a small percentage of the total soil strontium of a contaminated soil will be the Sr-90 isotope and that this isotope, unlike the stable isotope, will be more concentrated initially at the soil surface. Thus *Medicago sativa* might prove to be a better forage plant on Sr-90-contaminated soil than was indicated by the pot experiment, since it is normally deep-rooted.

The results indicate that animals grazing mixed legume-grass herbage, where the legume amounts to a significant proportion of the forage, will ingest far more Sr-90 than animals grazing all-grass pasture on equally contaminated soils. However, since the calcium content of milk is rather constant whether the animal is on a grass or a legume diet, the ratio of strontium to calcium of the diet becomes more important than the total amounts ingested. The advantage of the grass diet is that both the strontium and calcium levels are low to begin with, and a higher calcium supplement can be included to greatly decrease the Sr/Ca ratio.

As shown in the example in table 3, when a calcium supplement is added to a grass diet the strontium (or Sr-90) content of milk is reduced by a factor of 4.6. This factor is only 1.8 when the same amount of supplement is added to a legume diet. The strontium content of milk can be be reduced to about one third by using the same supplement with grasses rather than legumes. However, it should be possible to obtain an even greater reduction since more calcium could be added to a grass diet before calcium toxicity or phosphorus deficiency resulted. Thus, a factor of about 8 favoring calcium over Sr-90 seems reasonably obtainable by using a high-calcium supplement and a grass diet. This is in addition to the discriminatory factor of 7 to 10 obtained between the diet and the milk of the cow (Comar *et al.*, 1957).

The main practical implication of the results is that if the Sr-90 level in milk should ever rise to a level which might give cause for concern if maintained over a period of time, it would be desirable to restrict dairy cows to an all-grass diet with a high-calcium supplement. It should be stressed that such a high level of Sr-90 in milk is most unlikely to occur if the present level of atomic weapon testing is maintained, particularly as cows discriminate in favor of calcium as opposed to strontium. Nevertheless, sometime in the future it might prove desirable to take this measure to reduce the Sr-90 level in milk, particularly when consumed by children, although we may primarily visualize such measures as applicable to the local after-effects of atomic incidents. Such measures might first become necessary in regions of high rainfall—cf. radiostrontium content of human bone in regions of high rainfall and low rainfall (Bryant *et al.*, 1957; 1958)—or where other local factors predispose a higher than average radiostrontium content of milk. Such an area has been reported, with milk radiostrontium contents as high as 32 S.U. (United Press, 1958).

The hypothetical case of heavy localized Sr-90 fallout may be considered from two viewpoints. If the area affected is quite small it may be desirable to try to remove as much Sr-90 from the soil as possible, while if the area is too large for this to be practicable, as would generally be the case, the land must be farmed in a manner to reduce as much as possible the amount of Sr-90 ingested by humans and livestock. A content of 5×10^{-6} μc Sr-90/g has been suggested as a maximum permissible level of contamination for pasture herbage (Chamberlain *et al.*, 1955).

Current research on the removal of Sr-90 from soils includes removing the surface layer either mechanically or as an "asphalt cropsheet" (Anonymous, 1958; Schulz *et al.*, 1959). Another method might be to grow a heavy, shallow-rooted legume crop to be cut and removed from the ground, but in view of the results of Russell and Milbourn (1957) and Schulz *et al.*, (1959), it does not seem feasible to grow a crop on contaminated land for this purpose since only a very small percentage of the Sr-90 seems to be removed in one cropping.

In the case of Sr-90-contaminated land which must be maintained under farming conditions, a number of recommendations can safely be made.

On established pastures, immediately after the fallout, all existing herbage should be cut and removed. It has been found that little translocation of strontium occurs from leaves (Rediske and Selders, 1953; Martin, 1954;

Russell and Squire, 1958), therefore, one would expect little Sr-90 to be transferred to the roots from contaminated foliage. After removal of the herbage a heavy top dressing of nitrogenous fertilizer should be applied in order to stimulate the grasses at the expense of the clovers in a mixed legume-grass sward. As previously mentioned, *Medicago sativa* might be an acceptable legume because of its deep root system. Subsequently the foliage should be grazed by beef or dry cattle if at all possible, and a calcium supplement included in the diet. The interesting and plausible theory that much of the Sr-90 absorption in established swards is due to "stem-base" absorption (Russell, 1958) is a powerful reason for good grassland practices: the reduction of mat formation and the replacement of old swards by young pastures. It should be noted that the present work on the uptake of strontium from soil by forage species takes no account of the foliar absorption of radiostrontium which, in certain circumstances, might be more important than root absorption.

On bare fallow land the procedure would be to deep-plow, particularly when growing shallow-rooted species or when irrigating frequently, and sow one of the grasses most suited to the locality. Deep plowing has been shown to reduce Sr-90 uptake by certain species (Guliakin and Yudintsera, 1957; Russell and Milbourn, 1957, 1958). (Also see Schulz *et al.*, 1959.) No clover should be included in the mixture, and subsequent management should follow the lines previously indicated. Any soil deficiency of calcium should be corrected since it has been found (Romney *et al.*, 1959) that the uptake of radiostrontium on acid soil may be reduced by the application of calcium, though the addition of calcium to soils already adequately supplied is ineffective.

It may be hoped that such measures will not be necessary, but official concern is currently indicated by the publications (U.S.D.A., 1957; Ministry of Agr., 1958) of guidance for farmers in the event of heavy radioactive fallout due to nuclear warfare. As stated in these publications, farmers should consult local experts before attempting to reclaim contaminated crops or land.

This work was carried out primarily to investigate the possibility of strain differences within forage species so that selection might be made for low uptake of strontium. The small interspecific differences in strontium uptake within either the grasses or the legumes make intraspecific differences of any importance unlikely, and this is borne out by the results for the widely different strains of *Lolium perenne*. Nevertheless, the strains of *Trifolium repens* in the experiment show differential uptake of calcium, closely paralleled by the uptake of strontium. On the highly calcareous Nord soil, the uptake of calcium by Ladino, Dutch, and S.100 white clover is the same, but on the Yolo and Sacramento soils, with much less available calcium, S.100 took up substantially less calcium than the other two strains. It is, therefore, conceivable that at least within *T. repens*, it might be possible to select extreme individual plants for low uptake of calcium, which would also have a low uptake of strontium.

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Note: Russell and Garner (1959) have raised objections to the conclusions in our preliminary communication (Vose and Koontz, 1959) on the grounds that: (1) The ratio of Sr-90 to calcium in milk depends primarily on the ratio of Sr-90 to calcium in the total diet; (2) the present levels of Sr-90 in milk are due primarily to its lodging on the aerial tissues of plants and not to entry from the soil; and (3) pot experiments do not necessarily indicate conditions in the field due to the limitation on root depth, and suggest that deep-rooted species may have a lower ratio of Sr-90 to calcium.

These matters are dealt with in the present paper but the following points may be noted: (1) Although the ratio of Sr-90 to calcium in milk is dependent on the same ratio in the total diet, it is possible by giving supplement calcium in the diet to reduce the effective ratio in milk. As grass initially contains less strontium and calcium than does clover, a correspondingly greater reduction in ratio could be achieved by giving a calcium supplement to a grass diet. (2) While present levels of Sr-90 in milk may be due mainly to lodging on aerial tissues of plants, it is obvious that in the case of a radioactive element with a half life of 28 years a significantly active proportion of the element must ultimately enter the soil via the breakdown of vegetation, plowing, et cetera. (3) As Russell and Milbourn (1957) have shown that deep plowing reduces the amount of Sr-90 taken up by plants, we are surprised to find Russell and Garner (1959) so strongly advocating deep-rooted species, though we ourselves have recognized that well-established alfalfa, due to its extreme deep-rooting habit, may be a more valuable crop on Sr-90-contaminated land than was indicated in our experiment.

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